

# CLAIMS

1. An apparatus for use in conducting a chemical or biochemical reaction on a solid surface within an enclosed chamber, comprising:  
a substrate having a substantially planar surface with at least a portion of said surface representing a reaction area on which chemical or biochemical reactions are conducted;  
a plastic cover having a peripheral lip which sealingly contacts the substrate surface about the reaction area, wherein the cover and the reaction area form an enclosure having an interior space comprising a reaction chamber;  
a fastening means for immobilizing the cover on the substrate surface and providing a temporary, watertight seal between the cover and the reaction area; and  
a means for introducing fluid into the reaction chamber.
2. The apparatus of claim 1, wherein the reaction area is bound to or adapted to bind to molecular moieties.
3. The apparatus of claim 2, wherein the molecular moieties comprise molecular probes.
4. The apparatus of claim 1, further comprising a plurality of molecular probes bound to the substrate surface within the reaction area and arranged in a spatially defined and physically addressable manner.
5. The apparatus of claim 4, wherein the molecular probes are oligonucleotide probes.
6. The apparatus of claim 4, wherein the molecular probes are comprised of cDNA or PCR products.
7. The apparatus of claim 4, wherein the molecular probes are polypeptide probes.

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8. The apparatus of claim 1, wherein the cover is comprised of a material that is chemically and physically stable under conditions employed in hybridization assays.

9. The apparatus of claim 1, wherein the cover is comprised of a material that is thermally stable at temperatures of at least about 50°C.

10. The apparatus of claim 1, wherein the cover is comprised of a material that is chemically inert.

11. The apparatus of claim 1, wherein the cover is comprised of a material that is non-stick.

12. The apparatus of claim 1, wherein the cover is comprised of a material selected from the group consisting of polypropylene, polyethylene and acrylonitrile-butadiene-styrene.

13. The apparatus of claim 12, wherein the cover is comprised of polypropylene.

14. The apparatus of claim 1, wherein the substrate is comprised of glass.

15. The apparatus of claim 1, wherein the substrate is comprised of plastic.

16. The apparatus of claim 1, wherein the substrate is comprised of silicon.

17. The apparatus of claim 1, wherein the substrate is comprised of fused silica.

18. The apparatus of claim 1, wherein the dimensions of the cover, the peripheral lip, and the reaction area are such that the reaction chamber has a volume in the range of about 0.2  $\mu$ l to about 312  $\mu$ l.

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19. The apparatus of claim 18, wherein the reaction chamber has a volume in the range of about 1  $\mu$ l to about 200  $\mu$ l.

20. The apparatus of claim 18, wherein the reaction area is in the range of about 4  $\text{mm}^2$  to about 500  $\text{mm}^2$ .

21. The apparatus of claim 19, wherein the reaction area is in the range of about 20  $\text{mm}^2$  to about 350  $\text{mm}^2$ .

22. The apparatus of claim 1, wherein the means for introducing fluid into the reaction chamber comprises at least one port in the cover.

23. A device for conducting hybridization assays within an enclosed hybridization chamber, comprising:

a substrate having a surface with at least a portion of said surface representing a hybridization region;

a plurality of oligonucleotide probes bound to the substrate surface within the hybridization region and arranged in a spatially defined and physically addressable manner;

a cover which sealingly contacts the substrate surface about the hybridization region, wherein the cover and the hybridization region form an enclosure having an interior space comprising a hybridization chamber; and,

contained within the hybridization chamber, a sample fluid comprising a target molecule which may hybridize to a surface-bound molecular probe within the hybridization region, and wherein the sample fluid additionally comprises a surfactant of a type and present at a concentration effective to substantially reduce nonspecific binding and promote mixing of components within the sample fluid.

24. The device of claim 23, wherein the substrate surface is functionalized with a mixture of a first silane providing surface  $-\text{Si}-\text{R}^1$  groups where  $\text{R}^1$  is a chemically inert moiety

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and a second silane providing surface  $-\text{Si}(\text{L})_n-\text{R}^2$  groups where L is a linking group, n is 0 or 1, and  $\text{R}^2$  is a functional group enabling binding of the oligonucleotide probes.

25. The device of claim 23, wherein the cover is a plastic cover having a peripheral lip that contacts the substrate surface about the hybridization region.

26. The device of claim 23, wherein the cover is comprised of a material selected from the group consisting of polypropylene, polyethylene and acrylonitrile-butadiene-styrene.

27. The device of claim 26, wherein the substrate is comprised of glass.

28. The device of claim 26, wherein the substrate is comprised of plastic.

29. The device of claim 26, wherein the substrate is comprised of silicon.

30. The device of claim 26, wherein the substrate is comprised of fused silica.

31. The device of claim 23, further including a fastening means for immobilizing the cover on the substrate surface and providing a temporary, watertight seal between the cover and the hybridization chamber.

32. The device of claim 23, wherein the fluid further comprises a hybridization buffer.

33. The device of claim 31, wherein the hybridization chamber has a volume in the range of about 0.2  $\mu\text{l}$  to about 312  $\mu\text{l}$ .

34. The device of claim 31, wherein the hybridization chamber has a volume in the range of about 1  $\mu\text{l}$  to about 200  $\mu\text{l}$ .

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35. The device of claim 31, wherein the hybridization region has an area in the range of about 4 mm<sup>2</sup> to about 500 mm<sup>2</sup>.

36. The device of claim 32, wherein the hybridization region has an area in the range of about 20 mm<sup>2</sup> to about 350 mm<sup>2</sup>.

37. The device of claim 31, wherein the surfactant is selected from the group consisting of anionic surfactants, cationic surfactants, amphoteric surfactants, nonionic surfactants, and combinations thereof.

38. The device of claim 35, wherein the surfactant is an anionic surfactant.

39. The device of claim 36, wherein the anionic surfactant is a sodium, potassium, ammonium or lithium salt of lauryl sulfate.

40. The device of claim 36, wherein the anionic surfactant is lithium lauryl sulfate.

41. The device of claim 35, wherein the surfactant is a nonionic surfactant.

42. The device of claim 39, wherein the nonionic surfactant is polymeric.

43. The device of claim 42, wherein the nonionic surfactant is polyethylene oxide.

44. The device of claim 23, wherein the surfactant represents in the range of approximately 0.1 wt.% to 10 wt.% of the sample fluid.

45. The device of claim 44, wherein the surfactant represents in the range of approximately 0.5 wt.% to 5 wt.% of the sample fluid.

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46. The device of claim 45, wherein the surfactant represents in the range of approximately 0.75 wt.% to 5 wt.% of the sample fluid.

47. The device of claim 23, wherein the surfactant comprises a combination of polyethylene oxide and lithium lauryl sulfate, and further wherein the polyethylene oxide represents up to about 1 wt.% of the sample fluid and the lithium lauryl sulfate represents up to about 0.5 wt.% of the sample fluid.

48. The device of claim 23, wherein an air bubble is present within the hybridization chamber.

49. The device of claim 23, further including a fastening means for immobilizing the cover on the substrate surface and providing a temporary, watertight seal between the cover and the hybridization region.

50. The device of claim 49, further including a means for introducing fluid into the hybridization chamber.

51. A method for conducting a hybridization assay within an enclosed hybridization chamber, comprising:

(a) providing a device comprised of a (i) a substrate having a surface with at least a portion of said surface representing a hybridization region, wherein a plurality of oligonucleotide probes are bound to the substrate surface within the hybridization region and arranged in a spatially defined and physically addressable manner, and (ii) a cover which sealingly contacts the substrate surface about the hybridization region, wherein the cover and the hybridization region form an enclosure having an interior space comprising a hybridization chamber; and

(b) introducing into the hybridization chamber a sample fluid comprising (i) a target molecule which may hybridize to a surface-bound molecular probe within the hybridization

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region, (ii) a hybridization buffer, and (iii) a surfactant of a type and present at a concentration effective to substantially reduce nonspecific binding and promote mixing of components within the sample fluid; and

(c) maintaining hybridization conditions within the hybridization chamber for a period of time sufficient to allow hybridization between the target molecule and a surface-bound molecular probe to occur.

52. The method of claim 51, wherein the hybridization chamber has a volume in the range of about 0.2  $\mu\text{l}$  to about 312  $\mu\text{l}$ .

53. The method of claim 52, wherein the hybridization chamber has a volume in the range of about 1  $\mu\text{l}$  to about 200  $\mu\text{l}$ .

54. The method of claim 52, wherein the hybridization region has an area in the range of about 4  $\text{mm}^2$  to about 500  $\text{mm}^2$ .

55. The method of claim 53, wherein the hybridization region has an area in the range of about 20  $\text{mm}^2$  to about 350  $\text{mm}^2$ .

56. The method of claim 51, wherein the surfactant is selected from the group consisting of anionic surfactants, cationic surfactants, amphoteric surfactants, nonionic surfactants, and combinations thereof.

57. The method of claim 56, wherein the surfactant is an anionic surfactant.

58. The method of claim 57, wherein the anionic surfactant is a sodium, potassium, ammonium or lithium salt of lauryl sulfate.

59. The method of ~~claim 58~~, wherein the anionic surfactant is lithium lauryl sulfate.

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60. The method of claim 56, wherein the surfactant is a nonionic surfactant.
61. The method of claim 60, wherein the nonionic surfactant is polymeric.
62. The method of claim 61, wherein the nonionic surfactant is polyethylene oxide.
63. The method of claim 51, wherein the surfactant represents in the range of approximately 0.1 wt.% to 10 wt.% of the sample fluid.
64. The method of claim 63, wherein the surfactant represents in the range of approximately 0.5 wt.% to 5 wt.% of the sample fluid.
65. The method of claim 64, wherein the surfactant represents in the range of approximately 0.75 wt.% to 5 wt.% of the sample fluid.
66. The method of claim 51, wherein the surfactant comprises a combination of polyethylene oxide and lithium lauryl sulfate, and further wherein the polyethylene oxide represents up to about 1 wt.% of the sample fluid and the lithium lauryl sulfate represents up to about 0.5 wt.% of the sample fluid.
67. The method of claim 51, wherein an air bubble is present within the hybridization chamber.
68. A method of mixing a fluid in an enclosed chamber having a height less than approximately 0.5 mm, comprising the steps of:
  - (a) providing a chamber having a height of less than about 0.5 mm;
  - (b) introducing into the chamber (i) a fluid containing molecular components, and (ii) an air bubble;
  - (c) sealing the chamber; and

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(d) moving the chamber so as to create movement of the bubble within the fluid, whereby mixing of the molecular components within the fluid is effected by displacement of the fluid as the bubble moves within the chamber.

69. A kit for carrying out hybridization in an enclosed hybridization chamber, comprising:  
 a substrate having a surface with at least a portion of said surface representing a hybridization region;  
 a plurality of oligonucleotide probes bound to the substrate surface within the hybridization region;  
 a cover adapted to provide a sealed, enclosed chamber upon placement on the substrate surface over the hybridization region; and  
 surfactant effective to promote mixing and substantially reduce nonspecific binding in a hybridization assay conducted within the chamber.

70. The kit of claim 69, wherein the substrate surface is functionalized with a mixture of a first silane providing surface  $\text{-Si-R}^1$  groups where  $\text{R}^1$  is a chemically inert moiety and a second silane providing surface  $\text{-Si-(L)}_n\text{-R}^2$  groups where L is a linking group, n is 0 or 1, and  $\text{R}^2$  is a functional group enabling binding of the oligonucleotide probes.

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